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Isolation, culture, and lentiviral transduction of primary human T cells

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Engineering light-controllable CAR T cells for cancer immunotherapy

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Detailed protocol

Isolation, culture, and lentiviral transduction of primary human T cells

Protocol:

Isolation PBMCs from blood

Human peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats (San Diego Blood Bank) using Ficoll gradients (Amersham Biosciences).

1. Put 25 ml blood into a clean 50-ml tube, dilute with 25ml room temperature PBS (Sigma).

2. In a new 50-ml tube, first add 20 ml room temperature Ficoll (Amersham Biosciences) and slowly add 25 ml diluted blood dropwise without disturbing the interface between fico and blood.

3. Centrifuge at room temperature, 400 g for 15 min (set break to zero). Do not use brake function, otherwise the interface can be disturbed.

4. Discard the top layer plasma. Collect the middle yellowish layer PBMC into a new 50-ml tube.

5. Add 30 ml PBS to wash the collected PBMCs. Centrifuge 200 g, for 10 min at room temperature. Remove the supernatant and repeat the wash two more times.

CD3+ T cell isolation

CD3+ T cells were isolated from PBMCs using the Pan T cell isolation kit (Miltenyi). The protocol below was adapted from the manufacturer's instructions.

1. Gently resuspend cell pellet in 40 µL of MACS buffer (per 10^7total cells, same below).

2. Add 10 µL of Pan T Cell Biotin-Antibody Cocktail.

3. Mix well and incubate for 5 minutes at 4 °C.

4. After the incubation, add 30 µL of MACS buffer, mixed gently by pipetting, and then add 20 µL of Pan T Cell MicroBead Cocktail.

5. Mix well and incubate for 10 minutes at 4 °C.

6. After the incubation, add 500 uL MACS buffer and mix well.

7. Place LS Column (Miltenyi) in the magnetic field of a suitable MACS Separator (Miltenyi).

8. Prepare column by rinsing with 3 mL of MACS buffer.

9. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the CD3+ T cells.

10. Wash column with 3 mL of MACS buffer. Collect unlabeled CD3+ T cells that pass through, and combine with the cells collected from step 9.

11. Count the cell number. Centrifuge at 200 g for 10 min at room temperature. Remove the supernatant and resuspend the T cells in RPMI medium supplemented with 10%FBS/Pen/Strep/L-Glutamine, and 100 IU/ml IL-2 to the concentration of ~1 million/mL.

T cell activation

The isolated T cells were first activated for 48-72 hr using CD3/CD28-coated Dynabeads (Gibco) in RPMI medium supplemented with 10%FBS/Pen/Strep/L-Glutamine, and 100 IU/ml human IL-2.

1. Resuspend the Dynabeads in the vial by vortexing.

2. Transfer the desired volume of Dynabeads to a clean 1.5-mL tube (beads: T cells = 2:1).

3. Add 1 mL PBS to the beads, resuspend, and then place the tube on a magnet for 1 min and discard the supernatant.

4. Add the washed beads to T cells, mix gently. Culture in a humidified incubator at 37°C, 5% CO_2 for 2-3 days.

Lentiviral transduction

The cells were then transduced with concentrated lentivirus (or cocktail) at a multiplicity of infection of 10 for each virus by spinoculation on RetroNectin (Takara)–coated plates at 1800 g, 32 °C for 1 hr.

1. Wait 48-72 hr for T cells to proliferate. Then combine all T cells, and count the cells. Centrifuge down cells and resuspend to 2-6 million/ml in new growth medium.

2. Mix 75ul RetroNectin (Takara T100B) with 5ml PBS. Then add 0.8 ml in each well of non-tissue culture 6 well dishes. Make sure it covers all surface of each well. Wait for 2 hr at room temperature for the coating of RetroNectin on the dishes.

3. Remove RetroNectin, washed with PBS. Then mix 1.5ml lentivirus with 1-3 million PBMC in 0.5ml RPMI medium (2-6million/ml) and put all 2 ml mixture on each well of the 6 well dishes.

4. Centrifuge the cells in 6-well plates at 1800 g for 60 min at 32 °C. Then put the cells back into incubator.

5. Add 1ml growth medium in each well the next day and check the cells every day. If medium is yellow, add more medium.

6. After 4-5 days, the cells are ready for analysis and further experiments.

Solutions:

PBS: 0.01 M phosphate buffered saline, pH 7.4 at 25 °C.

MACS buffer:PBS supplemented with 0.5% bovine serum albumin, and 2 mM EDTA. Degas buffer before use.

T cell growth medium: RPMI1640 supplemented with 10% FBS, Pen/Strep (100 units/mL), 2 mM L-Glutamine, and 100 IU/ml human IL-2.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. wang, y. and Huang, Z. (2020). Isolation, culture, and lentiviral transduction of primary human T cells. Bio-protocol Preprint. bio-protocol.org/prep344.
2. Huang, Z., Wu, Y., Allen, M. E., Pan, Y., Kyriakakis, P., Lu, S., Chang, Y., Wang, X., Chien, S. and Wang, Y.(2020). Engineering light-controllable CAR T cells for cancer immunotherapy. Science Advances 6(8). DOI: 10.1126/sciadv.aay9209